CLONAL HYPERPLASIA OF CYCLIN D1+ MANTLE LYMPHOCYTES IN AN ASYMPTOMATIC PATIENT: A NEW ENTITY OR AN EARLY STAGE EVENT IN THE DEVELOPMENT OF A MANTLE CELL LYMPHOMA?

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ABSTRACT

Mantle cell lymphoma (MCL) is a B-cell neoplasm with a relatively aggressive clinical course. There is a subgroup of patients with histological, immunophenotypical and genetic characteristics corresponding to a MCL that present with atypical lymphocytes in peripheral blood (PB), with or without lymphocytosis, lymphadenopathy or splenomegaly and pursue a benign clinical course. In these cases, it is frequent to find mutated immunoglobulin heavy chain genes and lack of CD5 expression. In patients with lymphadenopathy, a mantle cell growth pattern is observed in the biopsy. It is uncertain whether these cases represent an early stage event in the development of MCL, an indolent form of MCL, or a newly recognized lymphoproliferative disorder not well characterized yet. We report an asymptomatic elderly patient who presented with a single submandibular adenopathy that was excised due to esthetic reasons. The adenopathy biopsy showed the presence of t(11;14)(q13;q32) and histology and immunophenotype consistent with a MCL. The abnormal lymphoid population was also detected in PB and in bone marrow. The patient has remained asymptomatic for five years without receiving any therapy. The existence of these asymptomatic cases with an indolent clinical course should induce to a strict clinical judgement in terms of terapeutic decisions.

INTRODUCTION

Mantle cell lymphoma (MCL) is a B-cell neoplasm that comprises 3-10% of non Hodgkin lymphomas. It occurs predominantly in middle aged to elderly individuals who present an advanced stage of the disease at diagnosis. Lymph nodes are the most frequently involved sites, while spleen, bone marrow, gastrointestinal tract or Waldeyer ring are other frequent localizations. The clinical behaviour of this entity is relatively aggressive, with poor response to conventional therapy (1). MCL is supposed to have its normal counterpart in a subset of naive pre-germinal center cells localized in primary follicles or in the mantle region of secondary follicles (2). Although it has been assumed that MCL bear unmutated variable inmunoglobulin heavy chain (IgV_H) genes (3), recent reports have shown the existence of somatic mutations in the IgV_H genes in a significant fraction of MCL patients (4, 5).

The involvement of secondary lymphoid organs is characterized by an architectural destruction due to a monomorphic lymphoid proliferation with a diffuse or vaguely nodular lymphoid proliferation, or mantle zone growth pattern (6). The majority of cases are composed of small to medium sized lymphoid cells with slightly to marked irregular nuclear contours, resembling centrocytes. The pattern of antigen expression in MCL is quite characteristic. B-lineage markers are positive. Surface immunoglobulins (Ig) are commonly IgM± IgD. Tumoral cells usually express CD5, CD43 and cyclin D1 (7) and are negative for CD10 and CD23. Some cases show CD43 or CD5 negativity (8) .By conventional cytogenetics and Southern blot analysis, nearly 75% of cases bear the t(11;14)(q13;q32) that juxtaposes the immunoglobulin heavy chain (IgH) and the Cyclin D1 (CCND1, PRAD1, BCL1) genes (9, 10) . However, when fluorescence in situ hybridization is applied, virtually all cases show this translocation (11, 12). The consequence of the t(11;14)(q13;q32) is an overexpression of Cyclin D1 which plays an important role in cell cycle regulation, inducing G1-S-phase transition by binding to cyclin-dependent kinases. Therefore, increased Cyclin D1 expression seems to contribute to lymphomagenesis in this type of tumor (13) but it has been shown that Cyclin D1 alone is not sufficient for the induction of B-cell lymphomas in mice (14).There is a very small subgroup of patients with a t(11;14) translocation that present with atypical lymphocytes in peripheral blood (PB), with or without

lymphocytosis, lymphadenopathy or splenomegaly and pursue a benign clinical course. In these cases, it is frequent to find mutated IgH genes and lack of CD5 expression. In patients with lymphadenopaty, a mantle cell growth pattern is observed in the biopsy. It is uncertain whether these cases represent an early stage event in the development of a MCL, an indolent form of MCL, or a newly recognized lymphoproliferative disorder not well characterized yet (15).

We report an asymptomatic elderly patient who presented with a single submandibular adenopathy that was excised due to esthetic reasons. The adenopathy biopsy showed histological, immunophenotypical and genetic characteristics corresponding to a MCL. The abnormal lymphoid population was also detected in PB and in bone marrow (BM). The patient has remained asymptomatic for five years without receiving any therapy.

CASE REPORT

In December 1999, a 70-year-old asymptomatic woman presented with an isolated submandibular lymphadenopathy of 18 months of evolution. On physical examination, no more lymphadenopathies nor organomegalies were detected and Waldeyer lymphatic ring was normal. Hematologic data showed: white blood cell count $8.08x10^9$/L (38.9% lymphocytes, absolute value $3,14x10^9$/L), Hb 138 g/L and platelet count $291x10^9$/L. Normal levels of LDH and β_2-microglobulin were detected.

MATERIALS AND METHODS

Histopathological and immunohistochemical studies

The lymph node biopsy was fixed in 10 % neutral buffered formalin and paraffin embedded for histological and immunohistochemical analysis. Immunohistochemical studies were performed in 3-4 μm thick sections. For immunohistochemical analysis, tissue sections were dried overnight at 60ºC, deparaffinized in xylene and hydrated. The DakoThechMate Immunostainer (DakoCytomation, Glostrup, Denmark) was used for immunohistochemical staining for the following antibodies: CD20 (L26), CD79 (JCB 117), CD3 (F 7.2.38), CD43 (DF-T1), IgD (Poli), IgM (Poli) (DakoCytomation), Bcl2 clone (100) (BioGenex, SanRamon, CA, USA) and CD7 (272), CD5 (4C7), CD4 (1F6), CD23 (1B12), CD10 (L26) and cyclin D1 (P2D11F11) (Novocastra,

NewCastle, UK). Heat induced epitope retrieval was used for all antigens except for IgM that was retrieved using enzyme digestion. The detection system employed was EnVision Plus (DakoCytomation).

Conventional Cytogenetics and Fluorescence in situ hybridization (FISH)

Chromosome analyses were carried out on lymphoid cells from phytohemagglutinin stimulated 72-hr lymph node, PB and BM cultures. Karyotypes were described according the International System for Human Cytogenetic Nomenclature (16). FISH analysis was performed on fixed cell proceeding from conventional cytogenetics culture and on paraffin embedded tissue sections of the lymph node using the BCL1/IgH dual color dual fusion locus specific probes (VYSIS, Downers Grove, IL) following the standard procedures.

Molecular Biology

Genomic DNA was extracted from proteinase-K treated PB leukocytes or BM cells by using the salting-out method (17). DNA from lymph node biopsy was obtained from two 15 micrometer sections of paraffin-embedded tissue using the QIAamp Tissue Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer instructions. DNA was amplified by polymerase chain reaction (PCR) with consensus primers specific for framework (FR)1, (FR)2, (FR)3 and Jh regions of the IgH gene. One of the primers was fluorescent-dye-labeled and analysis of the amplified products was performed by capillary electrophoresis in an automated DNA sequencer (ABIPrism 3100, Applied Biosystems, Foster City, CA). PCR amplifications were performed in duplicate. Mutational studies of IgV_H genes were performed using frozen lymph node tissue. Primers and PCR conditions were as previously described (18, 19).

RESULTS

Lymph node, peripheral blood and bone marrow, 1999

The lymph node biopsy revealed an alteration of the normal architecture due to the presence of numerous non-necrotizing well-demarcated granulomas. Between the granulomas, a lymphoid population composed by small T lymphocytes (CD7+, CD5+, CD3+) as well as primary and secondary follicles were observed. The primary follicles and the mantle zone of the secondary follicles were expanded by

a population of cyclin D1 positive small B lymphocytes (CD79a+, CD20+, CD23-, CD10-, IgD+, IgM+, CD5-, CD43- and bcl2+). The germinal center population did not show immunohistochemically abnormal findings. A PB morphologic examination detected a scarce B-lymphocyte population (3%), composed by atypical lymphocytes with condensed chromatin and scanty cytoplasm. No atypical cells were found in the BM aspirate. Flow cytometry immunophenotype of PB detected 12% of CD19+ cells with the same immunophenotype of the lymph node mantle cells, and monotypic expression of lambda light chain immunoglobulin.

Cytogenetic studies on fresh lymph node after 72-hour TPA stimulated cultures showed the presence of the following karyotype: 46,XX,t(11;14)(q13;q32)[6]/46,XX[14]. The translocation was assessed by FISH with a IgH/BCL-1 dual color dual fusion translocation probe. Twenty percent of the lymph node suspension cells were positive for the translocation, that was also detected in the mantle zone lymphocytes of the paraffin sections. In addition, conventional karyotypes were performed in PB and in BM, and t(11;14) was detected, as follows: 46,XX,t(11;14)(q13;q32)[5]/46,XX[19] in PB and 46,XX,t(11;14)(q13;q32)[1]/ 46,XX[42] in BM.

In the lymph node, IgH gene rearrangement analysis showed a clonal population, when analysing FR1 and FR2 regions, with mutated IgV_H genes (93.33% homology to VH2-70). PB and BM showed the same clonality pattern.

Taking into account that the patient had an isolated submandibular lymphadenopathy and that she was asymptomatic, the diagnosis of an indolent lymphoproliferative process with t(11;14) was established and a wait-and-see approach was taken.

Peripheral blood, 2002

With an absolute lymphocyte count of $2.5x10^9$/L, the PB morphologic examination did not show atypical lymphocytes. When flow cytometry immunophenotype was performed, 10% of cells with CD19+, CD20+, CD5-, CD10-, CD23- and FMC7- monoclonal B-cell population with monotypic lambda light chain expression was detected. Cytogenetic studies showed the following karyotype: 46,XX, t(11;14)(q13;q32)[3]/46,XX [17]. Clonality was also detected for framework regions (FR)1 and (FR)2 as at diagnosis.

Peripheral blood , 2003

The PB analysis showed an absolute lymphocyte count of $2.3x10^9$/L, with 2% of small lymphocytes with condensed chromatin and scanty cytoplasm. When flow cytometric immunophenotipic studies were applied, 9% of cells were CD19+, CD20+, CD5-, CD10-, CD23-, FMC7- and monotypic lambda light chain expression was found. The karyotype revealed a residual t(11;14) as follows: 46,XX,t(11;14)(q13;q32)[2]/46,XX[30]. IgH chain gene analysis detected the same clonality pattern as at diagnosis.

Peripheral blood, 2004

In the PB, $1.6x10^9$/L lymphocytes were detected, and among them 6% showed the same morphologic features previously found and 2% resembled centrocytes. Flow cytometry analysis revealed 31% of cells with the immunophenotypic characteristics found at diagnosis. Conventional cytogenetic studies showed 46,XX,t(11;14)(q13;q32)[1]/46,XX[39]. A clonal population was also detected by PCR for the IgH chain gene, showing the same pattern as at diagnosis.

The patient has remained asymptomatic for five years without receiving any therapy.

DISCUSSION

In the present case, the diagnosis of non-necrotizing granulomatous lymphadenitis would had been established if the lymph node had not entered a conventional cytogenetics training program. The lymph node karyotype adressed the subsequent studies. The lymph node biopsy was characterized by the presence of numerous non-necrotizing well-demarcated granulomas, that probably produced the increase in size which warned of the existence of the adenopathy. However, we have no evidence to be sure whether the granulomas were related or not to the abnormal lymphoid population. The patient did neither present any pathology in the organs that drain submandibular lymph nodes nor other lymphadenopathies or symptoms that could indicate a possible systemic granulomatous process. In the other hand, in the majority of lymphoproliferative disorders, including MCL, epitelioid granulomas can be found as a manifestation of a host response due to cytokine production by the tumoral cells or by the accompanying cells (20).

In the literature, a similar case to the herein presented patient has been reported (21). The diagnosis was established after the detection of atypical lymphocytes in PB, without an absolute lymphocytosis. This finding addressed the reevaluation of a lymphadenopathy, excised 8 years before, and initially diagnosed of follicular hyperplasia. In other similar cases, a peripheral lymphocytosis has been found without adenopathies, although, other patients presented splenomegaly (15). In these cases, treatment with high dose chemotherapy with stem cell rescue was considered, but because the patients were asymptomatic, a wait-and-see aproach was taken. All patients have remained stable.

The translocation t(11;14) is quite characteristic of MCL. It has never been detected in healthy individuals using techniques of low and medium sensitivity, such as conventional cytogenetics or FISH. By applying very sensitive techniques, like real time PCR, to test the presence of t(11;14) in PB samples from healthy volunteers, the finding of this translocation is very rare, with a very low prevalence (only 1.1% of cases showed 6 copies among 10^6 PB mononuclear cells) (22). Translocation t(11;14) has also been described in other entities different from MCL. It is widely accepted that t(11;14)(q13;q32) is found in some cases of multiple myeloma. Not free of controversy, this translocation has been also described in agressive and atypical B-chronic lymphocytic leukemia (B-CLL), in splenic marginal zone B-cell lymphoma (SMZBCL) and in B-cell prolymphocytic leukemia (B-PLL) (23-25). Later studies have concluded that many, but not all, of those patients were MCL cases that had not been previously well classified (26, 27).

In MCL, a 25% of cases show IgV_H somatic mutations. The presence of IgV_H somatic mutations has been strongly associated with a better prognosis in CLL (28), but no statistically significant differences have been found when the association with prognosis has been analyzed in MCL (5). In the other hand, in the present case and in the majority of indolent lymphoproliferative disorders with t(11;14) the presence of IgV_H somatic mutations has been detected.

Between 10 to 20% of MCL cases are CD5 negative. In two different MCL series of 76 patients (18/76 CD5-) and 58 patients (9/58 CD5-) (5, 29), CD5 expression did not distinguish different overall survival probabilities among the patients. Anyway, the present case and the majority of indolent lymphoproliferative disorders with t(11;14) are CD5-.

Therefore, CD5 negative MCL or MCL with somatic hypermutations of the IgV_H gene do not show a different clinical behaviour than CD5 positive MCL. In the other hand, it is noteworthy the existence of a small group of patients with t(11;14), CD5 negativity and the presence of somatic hypermutations with an indolent clinical course. At present, we do not have enough data to determine if these asymptomatic cases are an indolent form of MCL, that would represent a redefinition of some aspects of this entity, or correspond to an indolent lymphoproliferative disorder still not well characterized.

In conclusion, the existence of asymptomatic cases with morphologic, immunohistochemical and cytogenetic characteristics similar to those of classical MCL but with an indolent clinical course should induce to a strict clinical judgement in terms of therapeutic decisions.

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